Cardiovascular Risk Factors for Calcific Aortic Valve Disease

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Introduction

Over the past decade epidemiological studies have revealed the risk factors associated for vascular atherosclerosis, including male gender, smoking, hypertension and elevated serum cholesterol, are similar to the risk factors associated with development of aortic valve stenosis. There is also growing evidence that renal failure (RF) is responsible for accelerated vascular calcification. These clinical studies demonstrate that defining these risk factors for this disease may delineate preventive strategies to slow progression and to possibly modify the disease process. In summary, these findings suggest that medical therapies may have a potential role in patients in the early stages of this disease process to slow the progression to severe calcific aortic valve disease and delay the timing to intervention.

With the decline incidence of rheumatic carditis, calcific aortic stenosis (AS) has become the most common indication for surgical valve replacement in the US. Numerous epidemiologic studies identified risk factors for AS disease development, which are similar to those of vascular atherosclerosis, including smoking, male gender, body mass index, hypertension, elevated lipid and inflammatory markers, metabolic syndrome and renal failure (Deutscher et al. 1984; Hoagland et al. 1985; Aronow et al. 2001; Mohler et al. 1991; Lindroos et al. 1994; Boon et al. 1997; Chui et al. 2001; Wilmshurst et al. 1997; Chan et al. 2001; Briand et al. 2006; Palta et al. 2000; Peltier et al. 2003; Stewart et al. 1997; Otto et al. 1999; Faggiano et al. 2006; Pohle et al. 2001).

Aortic Valve Cardiovascular Risk Factors

Stewart et al. (1997, 1999), described the risk factors for calcific AS identified in the Cardiovascular Health Study. The investigators examined 5,621 patients older than the age of 65 years found by Doppler Echocardiography that the prevalence of aortic valve sclerosis was 29% and AS was 2% in this population. The investigators demonstrated that the clinical risk factors important for the development of atherosclerosis are also the independent risk factors for AS including age, male gender, height (inverse relationship), history of hypertension, smoking and elevated serum levels of lipoprotein(a) and LDL levels (Stewart et al. 1997).

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Data from several studies have confirmed that all of these traditional risk factors including metabolic syndrome (Briand et al. 2006), and RF (Palta et al. 2000), which are important in the development of vascular atherosclerosis, are also implicated in the development of calcific AS. These findings provide the foundation to study targeted strategies for medical therapy, including for example, medications for hyperlipidemia, hypertension and diabetes. There are a growing number of experimental in vivo models of calcific AS which demonstrate primarily that lipids (Rajamannan et al. 2001, 2002; Drolet et al. 2003, 2006; Weiss et al. 2006; Aikawa et al. 2007; Shao et al. 2005), diabetes (Shao et al. 2005) and RF (Shuvy et al. 2008) are important in the development of this disease. Early studies have demonstrated that cholesterol (Ortlepp et al. 2006), and Vitamin D (Drolet et al. 2003), can induce early stenosis of the valve (Drolet et al. 2003) as documented by echocardiographic measurements.

Lipids and other cardiovascular risk factors induce oxidative stress (Weiss et al. 2006; Rajamannan et al. 2005a; Miller et al. 2008) in the aortic valve endothelium similar to vascular endothelium (Wilcox et al. 1997) which in turn activates the secretion of cytokines and growth factors important in cell signaling. The early atherosclerotic and abnormal oxidative stress environment also plays a role in the activation of the calcification process in the myofibroblast cell. The signaling molecules important in the development of vascular atherosclerosis are also important in the development of valve calcification including: MMP (Kaden et al. 2004a; Jian et al. 2001), Interleukin 1 (Kaden et al. 2003), transforming growth factor-beta(TGF-beta) (Jian et al. 2003), purine nucleotides (Osman et al. 2006a, b), RANK (Kaden et al. 2004b), osteoprotegrin(OPG) (Kaden et al. 2004b), elastolytic cathepsins S, K, and V and their inhibitor Cystatin C in stenotic aortic valves (Helske et al. 2006) Toll-like receptors (Yang et al. 2009), TNF alpha (Kaden et al. 2005), MAP Kinase (Gu and Masters 2009) and the canonical Wnt pathway (Shao et al. 2005; Rajamannan et al. 2005b; Caira et al. 2006). Similar to vascular atherosclerosis these events are potential cellular targets for pharmacologic agents to slow this disease process.

Renal Failure as a Risk Factor Associated with Calcific Aortic Valve Disease

Cardiovascular disease is the leading cause of mortality in patients with renal disease and is attributed to both traditional and non-traditional cardiovascular risk factors. One of the most devastating complications in this population is ectopic calcification. Ectopic calcification is defined as inappropriate biomineralization occurring in soft tissues as a result of systemic mineral and hormonal imbalance (Giachelli 2004; Goodman 2001). Aortic valve is one of the most important tissues which are involved in the calcification process.

The prevalence and extent of AS in this population of patients is poorly explained by traditional cardiovascular risk factors (Moe 2004; Yao et al. 2004) abnormalities of mineral metabolism are likely to contribute to AS development and progression. Contrary to "senile AS", patients with RF associated AS are characterized by significant mineral disturbances especially involving phosphate and calcium metabolism (Kalpakian and Mehrotra 2007; Tomson 2003). Most of these patients develop hyperphosphatemia as well as an increased Ca – phosphate product levels (Verberckmoes et al. 2007). Calcium-phosphorus product is associated with increased ectopic calcification and cardiovascular morbidity and mortality (Cozzolino et al. 2001).

Phosphorus excess is an independent cardiovascular risk factor for morbidity and mortality in patients with advanced RF (Kestenbaum et al. 2005; Menon et al. 2005) as well as in normal subjects. In addition to the effects of phosphate during passive mineralization, recent data suggest that phosphate induces calcification by activating osteoblast transformation in vascular smooth muscle cells (VSMC) (Giachelli 2003). Elevated phosphate level is a key element in activation of osteoblast specific maturation factors. Although the exact mechanism is still unknown, this effect seems to be

mediated by a sodium-dependent phosphate co-transporter, Pit-1 (Glvr-1) (Giachelli 2003). In vitro studies in VSMC cells demonstrated that inhibition of phosphate uptake abolished calcification. The specific role of phosphate in RF associated AS is still under investigation, data obtained from animal study suggest that hyperphosphatemia and elevated parathyroid hormone (PTH) rather than uremia itself are the mediator of AS (Shuvy et al. 2008).

Parathyroid hormone is the most important regulator of calcium and phosphate metabolism (Goodman 2005). It is essential for both bone formation and osteoblast activity, and increases the conversion of vitamin D to its active metabolite (Murray et al. 2005). Hyperparathyroidism is often accompanied with hyperphosphatemia making the evaluation of the specific effect of PTH on calcification difficult. Nevertheless animal studies find the PTH induces ectopic calcification, which is unrelated to the serum levels of calcium and phosphate. The mechanism of this phenomenon is unclear and it may be related to elevated bone turnover (Neves et al. 2007). The role of calcium in the pathogenesis of AS is less established, and no significant increased progression of AS was found in women taking oral calcium supplementation (Bhakta et al. 2009).

Apart of mineral and hormonal disturbances numerous additional factors as oxidative stress, malnutrition, endothelial dysfunction and constant low-grade inflammation are common in that population (Pecoits-Filho et al. 2002). Available data suggest that pro-inflammatory cytokines play a central role in the genesis of both malnutrition and vascular disease in RF (Stenvinkel et al. 2005). Strong associations between malnutrition, inflammation and atherosclerosis suggest the presence of a syndrome – malnutrition, inflammation, and atherosclerosis (MIA), which is associated cardiovascular morbidity.

Calcification Inhibitors

Although the majority of patients with significant RF develop ectopic calcification, not all of them have vascular calcification, naturally occurring inhibitors of calcification may be involved in this phenomenon. Fetuin-A (alpha-Heremans–Schmid glycoprotein), a 59 kDa glycoprotein, consisting of two cystatin-like domains and a smaller unrelated domain, is predominantly synthesized in liver (Westenfeld et al. 2009). It is secreted into the blood stream and deposited as a noncollagenous protein in mineralized bones. Fetuin-A binds calcium phosphate (Heiss et al. 2003), and thus directly prevents calcium-phosphate to cause ectopic calcification (Westenfeld et al. 2009). Dialysis patients have significantly reduced serum fetuin-A levels compared with control subjects (Ketteler et al. 2003). Interestingly, Ketteler et al. reported an inverse relationship between serum fetuin-A and C-reactive protein serum in dialysis patients, implying that inflammation decreases fetuin-A level, furthermore, its level is significantly decreased in patients with major components of the MIA syndrome (Wang et al. 2005). The role of futin A in preventing AS was demonstrated in RF population. This study demonstrates in patients with low serum fetuin-A have the greatest prevalence of valvular calcification and 0.01 g/l increase in serum fetuin-A is associated with a 6% decrease in the risk of valvular calcification (Wang et al. 2005). Recently low fetuin A level were found in patients with senile AS (Koos et al. 2009).

Matrix Gla protein (MGP) is one of three vitamin-K dependent proteins have been isolated in bones. MGP inhibit calcification via modulation of bone morphogenic protein-2 (BMP-2) activity, which is known to induce calcification. Warfarin treatment decreases MGP levels and may increase calcification, actually several clinical and experimental (Price et al. 1998) studies support this hypothesis. In patients undergoing valvular replacement or in patients with renal failure, warfarin treatment was associated with greater valvular calcification (Zebboudj et al. 2003). An addition protein that requires vitamin k and decreased during warfarin treatment, is the product of the gene growth arrest-specific 6 (GAS6) (Nagata et al. 1996) which prevents apoptosis and calcification in VSMC. Phosphate is a negative modulator of GAS6, therefore hyperphosphatemia promotes apoptosis and calcification (Son et al. 2006).

Osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) superfamily of proteins, is involved in bone remodeling as well as ecotpic calcification, through its action as a decoy receptor for RANKL (Bennett et al. 2006). The exact role of the RANK-RANKL complex in the calcification process is not clear. It was shown to trigger and osteoclast differentiation and which are highly important in the calcification process. The importance of this pathway is demonstrated in OPG-deficient mice which develop severe osteoporosis, as well as ectopic calcification; administration of OPG reduces this calcification (Bucay et al. 1998). The role of RANK-RANKL-OPG was shown both in vitro and in vivo, especially in the pathogenesis of renal failure associated calcification. As opposed to fetuin-A, OPG plasma levels are increased in patients with significant calcification, furthermore high level of OPG may predict cardiovascular calcification in RF population (Morena et al. 2009). This unique observation may be explained that OPG is protective and its elevation in a compensation in patients with extensive disease (Schoppet et al. 2002). The specific role RANK-RANKL-OPG was demonstrated in a recent study showing high expression of RANKL in human calcified aortic valves (Kaden et al. 2004b), and in animal model of RF associated valve calcification (Shuvy et al. 2008).

Pathogenesis of Renal Failure Associated Valve Calcification

Vascular and valve calcification is considered to be an organized, regulated process comparable to bone mineralization which involves trans-differentiation of valvular myofibroblasts into osteoblasts. The presence of various components associated with bone mineralization such as bone specific proteins in valvular lesions supports this concept. There are three phases necessary for the myofibroblast cell to differentiate to form bone. These phases include first: activation of cell proliferation, second: extracellular matrix synthesis, third: mineralization of the bone formation. Several RF associated mediators are involved in this process: Activation of PTH receptor induces

several osteoblast transcription factors (e.g. Runx-2) and proteins (e.g. osteopontin and osteocalcin) that stimulate osteoblast maturation. Runx-2 is crucial in the differentiation of mesenchymal cells to an osteoblastic phenotype, a process that may contribute to AS. Osteopontin and osteocalcin are the most abundant glycoproteins produced by osteoblasts, which compose the organic part of the bone and are essential for calcification. Hyperphosphatemia is involved in several phases of calcification: The final step in the mineralization process for bone formation is apoptosis. The presence of apoptosis is critical for bone mineralization. Apoptosis is the final common pathway necessary for the transition of the osteoblasts to mineralized bone. Phosphate induces osteoblast differentiation and apoptosis of vascular smooth muscle cells, resulting in calcification. Furthermore, the pro-apoptotic effect of phosphate is mediated through inhibition of survival pathways.

Conclusion

Most of the risk factors for AS are identical with the risk factors for atherosclerosis and may be targeted in patients with AS. However, it seems that the medical intervention in valve calcification must take place in very early stages of the disease and may be ineffective later. A possible explanation is that in early stages of the disease the inflammatory features are more prominent, while in later stages calcification and bone formation are dominant. The exact timing of medical therapy is highly important as different processes are involved in the course of the disease. Furthermore, defining the mechanistic domain in every phase and finding specific markers for disease progression are the foundation to possible therapeutic interventions to counter AS. The complexity of AS is illustrated in patients with RF.

Renal failure is a major risk factor for AS and patients with RF associated calcification have more severe and rapidly progressive disease than patients with "senile AS". Renal failure associated calcification is a complex process involving different pathways than

"senile AS". Apart from the traditional risk factors and the importance of atherosclerosis, specific unique metabolic conditions as hyperphosphatemia, elevated PTH and decreased calcification inhibitors play an important role. Due to the difference in the pathogenesis of RF associated AS, RF population may react differently to medical intervention and therefore any intervention should be evaluated specifically in the renal failure milieu. Although patients with RF often have more advanced disease, targeting the metabolic abnormalities such is decreasing serum phosphate levels, or preventing hyperparathyroidism, in early stages may halt the rapid course of AS.

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